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Prostaglandin levels and lysosomal enzyme activities in irradiated rats

P. J. Trocha

G. N. Catravas

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BETHESDA, MARYLAND 20014

REVIEWED AND APPROVED

GEORGE N. CATRAVAS, D.Sc.
Chairman
Biochemistry Department

PAUL E. TYLER, M.D.
CAPT, MC, USN
Director

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20. ABSTRACT (continued)

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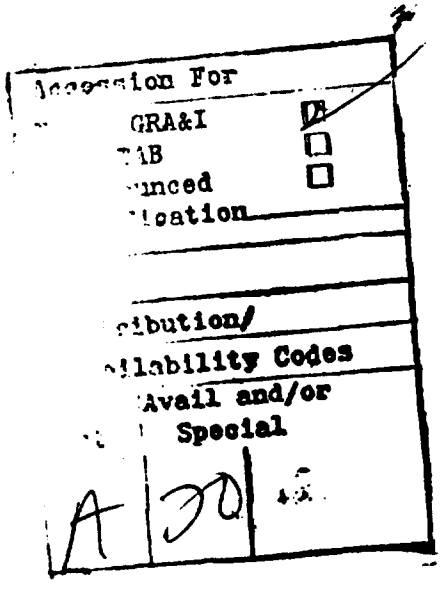

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Prostaglandin levels and lysosomal enzyme activities in irradiated rats†

P. J. TROCHA and G. N. CATRAVAS

Biochemistry Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014, U.S.A.

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Whole-body irradiation of rats results in the release of hydrolases from lysosomes, an increase in lysosomal enzyme activities, and changes in the prostaglandin levels in spleen and liver tissues. A transient increase in the concentration of prostaglandins E and F and leakage of lysosomal hydrolases occurred in both spleen and liver tissues 3–6 hours after the animals were irradiated. Maximal values for hydrolase activities, prostaglandin E and F content, and release of lysosomal enzymes were found 4 days postirradiation in rat spleens whereas in the liver only slight increases were observed at this time period for prostaglandin F levels. On day 7 there was a final rise in the spleen's prostaglandin E and F concentrations and leakage of hydrolases from the lysosomes before returning to near normal values on day 11. The prostaglandin F concentration in liver was also slightly elevated on the 7th day after irradiation and then decreased to control levels.

1. Introduction

Recent evidence suggests that ionizing radiation affects the prostaglandin levels in animal tissues (Eisen and Walker 1976, Păusescu, Chirvasie, Teodosiu and Păun 1976, Pryanishnikova, Zhulanova and Romantsev 1978). These changes in prostaglandin levels have been found to increase within several hours after exposure of the animal and to remain elevated for days, especially in the radiosensitive spleen and thymus tissues (Eisen and Walker 1976).

Additional evidence has been accumulating to show that synthesis and leakage of enzymes from lysosomes are also affected by radiation (Watkins 1975). Lysosomal hydrolases and proteinases have been found to increase and reach abnormally high levels in many mammalian tissues several days after radiation treatment (Snyder 1977).

Previous studies (Eisen and Walker 1976, Păusescu *et al.* 1976, Pryanishnikova *et al.* 1978, Watkins 1975, Snyder 1977) suggest that both prostaglandins and lysosomes are involved with a mechanism that controls the inflammatory process in tissues injured by ionizing radiation. Other investigators (Ignarro 1975) have tried to implicate prostaglandins and lysosomes in the tissue injury process. But much considerable controversy continues as to whether prostaglandins are actually involved with the stability of lysosomal membranes, since these studies used *in vitro* environments (Ignarro 1975). Therefore, this investigation was performed using an

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in vivo system to determine if prostaglandins and lysosomes are influenced by ionizing radiation. If they are interrelated, then the effect of prostaglandins on the integrity of the lysosomal membrane can be determined.

2. Materials and methods

2.1. *Chemicals*

Phenolphthalein β-glucuronide, 4-methylaminophenol, glycero-2-phosphate, and silicic acid (Sil B-200) were obtained from Sigma Chemical Co., St Louis, MO. Amersham (Arlington Heights, IL) suppplied [5,6(n)-^{3}H] prostaglandin E_1 and [5,6,8,11,12,14,15(n)-^{3}H]prostaglandin $F_{2\alpha}$ for tracer studies. ^{3}H prostaglandin E and $F_{2\alpha}$ RIA kits were purchased from Clinical Assays, Inc., Cambridge, MA.

2.2. *Animals*

A total of 750 male Sprague-Dawley rats, 75–150 g, were used throughout the investigation. They were divided into three groups of 250 each, in which two rats per cage were kept in a room maintained on a 12-hour light (0600–1800) and 12-hour dark (1800–0600) cycle. All the animals were given a Wayne Lab Blox diet and water *ad libitum*.

2.3. *Irradiation of animals*

From each group, 190 rats were placed in Plexiglas restrainers and exposed bilaterally to 1000 rad of ^{60}Co radiation at a dose rate of 500 rad/min. The remaining 60 rats were kept as sham controls. At designated time intervals after irradiation, 12–15 exposed and 4 sham-irradiated control rats were sacrificed by exsanguination under ether anaesthesia.

2.4. *Preparation of tissue homogenates and enzyme assays*

Spleens and livers were excised from rats and frozen in liquid nitrogen except for 0·05–0·1 g of tissues from each organ. The portions of the organs that were quickly frozen in liquid nitrogen were later used for assaying prostaglandin concentrations. The fresh sections of spleen or liver tissue were gently homogenized in 2 ml of 0·2 M KCL using a Dounce homogenizer. One half of each homogenate was centrifuged at 12 000 g in a refrigerated centrifuge for 10 min in order to obtain a supernatant free of lysosomes. The remaining half was frozen and thawed twice. The lysosome-free supernatants and thawed whole homogenates were then assayed for acid phosphatase and B-glucuronidase activity (Barrett and Heath 1977).

2.5. *Isolation and purification of prostaglandins*

Tissues from rats frozen in liquid nitrogen were weighed, homogenized, and the prostaglandins extracted no later than 1 hour after the animals were sacrificed using a modification of the method employed by Jouvenaz, Nugteren, Beerthuis, and Van Dorp (1970) as shown in figure 1. Each liver sample, weighing 3–4 g was quickly homogenized in ice-cold ethanol containing ^{3}H labelled prostaglandin E or F tracer for 30 s with a Polytron (Brinkmann Instruments, Inc., Westbury, NY). However, it was necessary to pool four or five spleens to obtain an adequate amount of spleen tissue (0·5–3 g) for each prostaglandin determination. This method results in a 30–55 per cent recovery of prostaglandins found in rat tissue. Corrections for prostaglandin losses in each sample were made when calculating their concentrations.

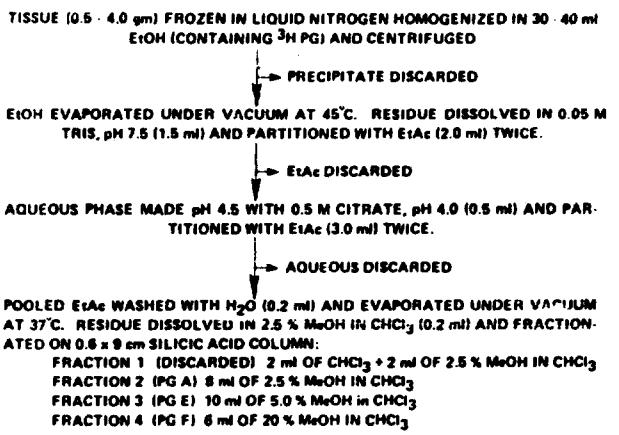

Figure 1. Scheme for isolating prostaglandins.

A comparison of our procedure (figure 1) with others in which indomethacin (Vane 1971), or acid (Hensby 1977), was included in the homogenization media was made in order to determine if any artifactual prostaglandin synthesis occurred during its isolation. It was found that after correcting for losses, the concentration of prostaglandins isolated from rat tissues was essentially the same when using either ethanol or homogenation media which contained indomethacin or acid. In an additional study rats injected with indomethacin (10 mg/kg wt. of animal) 2 hours prior to their sacrifice had prostaglandin concentrations the same as the untreated controls when analysed by the method shown in figure 1. Therefore, employment of our technique allows the determination of the *in vivo* levels of prostaglandin in rat spleen and liver tissues.

2.6. *Assays of prostaglandins*

Analysis of purified prostaglandins E and F was performed by immunoassay (Jaffe and Behrman 1974). The binding characteristics as well as the degree of prostaglandin cross reactivity for the commercially available antisera that was used in this study have been previously determined by Jaffe and Behrman (1974) and Levine, Gutierrez Cernosek and Van Vunakis (1971).

2.7. *Statistics*

Statistical analyses were based on the mean that was calculated from the average values of three separate experiments. Therefore, a total of 12 control and 36–45 experimental animals were used per time interval for calculating the appropriate means and standard errors. Differences between experimental and corresponding control values for the same time interval were considered significant if Student's unpaired t test gave a probability (p) of less than 0·05. The experiments were monitored for a period of 11 days.

3. Results

3.1. *Liver lysosomal enzyme activities*

β-glucuronidase activity was measured by the release of phenolphthalein from the substrate phenolphthalein-β-glucuronide. As shown in figure 2, the β-glucuronidase activity of the enzyme was not significantly different in the irradiated rats as compared to the corresponding nonirradiated controls, although a slight but

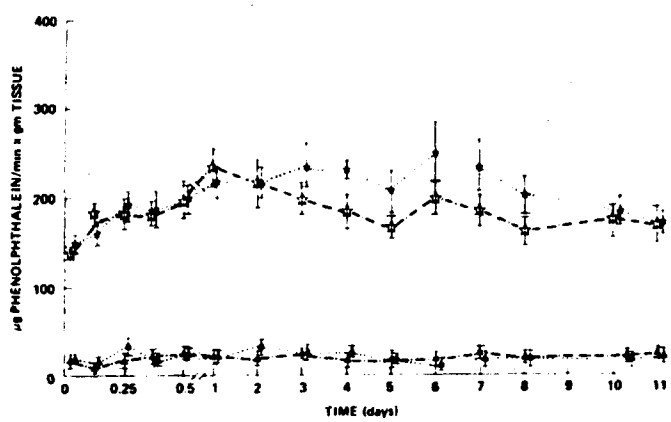

Figure 2. Effect of ^{60}Co radiation on liver β-glucuronidase activity in rats. ···★··· = unfractionated liver experimental; - - - - - = unfractionated liver control; ···▲··· = 12 000 g liver supernatant experimental fraction; - - -△- - - = 12 000 g liver supernatant control fraction. Means ± S.E. S.E. indicated by vertical lines. $p > 0{\cdot}05$ (toward corresponding control) for all time intervals. $n = 9$.

insignificant increase was observed 3–8 days after exposure. β-glucuronidase activities in the lysosome-free 12 000 g supernatants from the experimental and the control liver-tissue homogenates also showed no changes (figure 2) except for a small nonsignificant elevation at 6 hours postirradiation ($p = 0{\cdot}19$). No significant changes in acid phosphatase levels in the liver samples were observed following ionizing radiation (data not shown).

3.2. *Spleen lysosomal activities*

An increase in the lysosomal β-glucuronidase activity was observed in the spleen following irradiation. Within 3–6 hours after exposure, its activity began to rise, reaching a maximal level in 3–4 days, which was four times greater than the control levels (figure 3). After day 4 the enzyme gradually decreased until day 10, when it

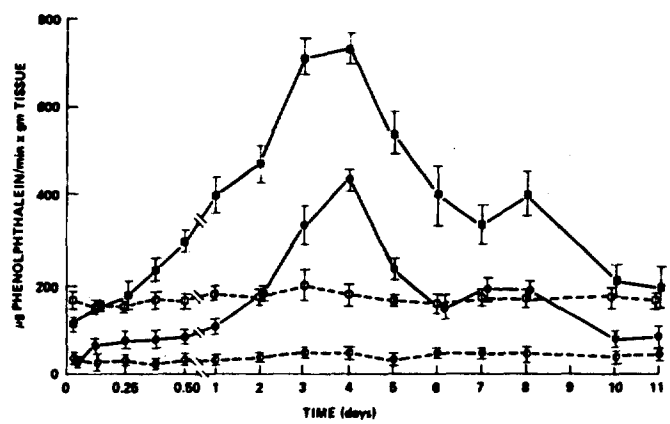

Figure 3. Effect of ^{60}Co gamma radiation on spleen β-glucuronidase activity in rats. ——■—— = unfractionated spleen experimental; - - -□- - - = unfractionated spleen control; ——●—— = 12 000 g spleen supernatant experimental fraction; - -○- - = 12 000 g spleen supernatant control fraction. Means ± S.E. $p \leqslant 0{\cdot}05$ (toward corresponding control) for 0·5- to 8-day unfractionated samples. $p \leqslant 0{\cdot}05$ (toward corresponding control) for 1- to 8-day 12 000 g supernatant samples. $n = 9$.

returned to a normal physiological level. Similar increases were observed with acid phosphatase activity.

Figure 3 also shows that the β-glucuronidase activity level in the lysosome-free homogenates (12 000 g supernatants) rose insignificantly within 3–6 hours (3 hours, $p=0{\cdot}26$; 6 hours, $p=0{\cdot}08$) after irradiation of the animals and continued to increase until day 4. The activity then slowly decreased to near control levels. Similar patterns for acid phosphatase activities were found.

3.3. *Percentage of soluble lysosomal enzyme activities*

Figure 4 illustrates the percentage of soluble β-glucuronidase (12 000 g supernatant) found in the unfractionated homogenates. Within 3–6 hours after irradiation of the animal, the percentages of soluble enzymes released from the liver and spleen lysosomes were significantly elevated 1·5- to 1·8-fold (6 hours, $p=0{\cdot}05$; 3 and 6 hours spleen, $p<0{\cdot}01$) as compared with the negligible increases in total tissue enzyme activities. An approximate twofold increase in soluble acid phosphatase activity was also observed in spleen and liver at this time period. On days 3–5 and 7–8, a pronounced increase in soluble β-glucuronidase was observed in the spleen but not in liver supernatants. Acid phosphatase activities again showed similar changes for the spleen at 3–5 and 7–8 days postirradiation (their patterns are not shown in the figure).

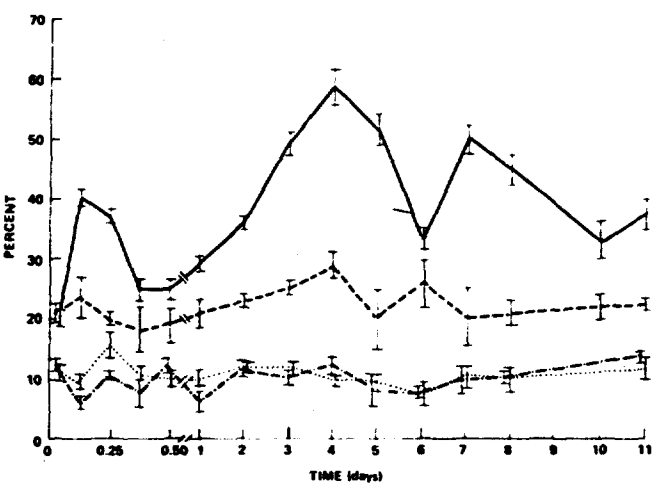

Figure 4. Effect of ^{60}Co gamma radiation on the percentage of soluble β-glucuronidase activity in rat tissue supernatants devoid of lysosomes. ——— = spleen experimental; - - - - = spleen control; ····· = liver experimental; - · - · - · = liver control. Means ± S.E. $p\leqslant 0{\cdot}05$ (toward corresponding spleen control) for 0·125-day, 0·25-day, 2- to 5-day, and 7- to 11-day spleen samples. $p\leqslant 0{\cdot}05$ (toward corresponding liver control) for 0·25-day liver sample. $n=9$.

3.4. *Prostaglandin levels in liver*

Figure 5 illustrates a two- to four-fold increase in both PGE and PGF levels at 3–6 hours after gamma irradiation of the whole animal. By hour 9, both the prostaglandins had returned to normal levels. For the remaining 10 days the PGE concentration in the liver was normal. The PGF values, however, rose to a maximal value on day 4 after exposure, decreased to near control levels on day 6, and increased a third time to a maximal level on day 7. Concentration of PGF then decreased to a slightly elevated value on day 11.

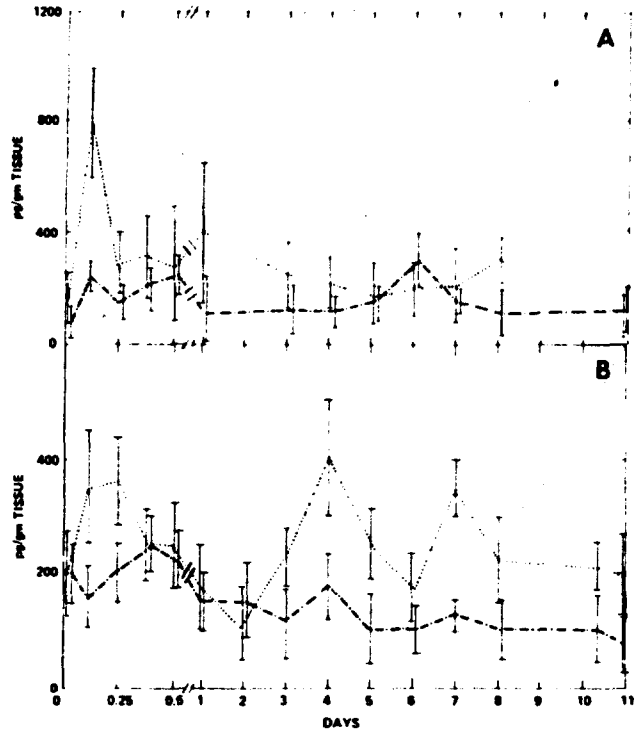

Figure 5. Effect of ^{60}Co gamma radiation on the prostaglandins in rat liver. PGE values illustrated in (A); PGF concentrations shown in (B). ·····=experimental; - - - - - -=control. Means ± S.E. $p \leqslant 0{\cdot}05$ (toward corresponding liver control in (A) for 0·125-day PGE samples. $p \leqslant 0{\cdot}05$ (toward corresponding liver control in (B) for 0·125-day, 0·25-day, 4-day and 7-day PGE samples. $n = 12$.

3.5. *Prostaglandin levels in spleen*

The radiosensitive spleen displayed very pronounced changes in prostaglandin levels. As shown in figure 6, a sharp transient increase in both prostaglandins E and F occurred 3 hours after exposure of the animal. The levels then decreased to near control values for PGE and somewhat elevated levels for PGF. The prostaglandin content in the spleen tissue again started to increase 3 days after irradiation treatment. It reached on day 4 an extreme value that was seven times greater than the normal concentration before quickly dropping to a lower concentration on the fifth day. On the seventh day following irradiation, a third maximum was observed for PGE and PGF values, which slowly decreased to near normal levels on the eleventh day after exposure.

4. Discussion

4.1. *Relationship between lysosomes and prostaglandins*

The results show that ionizing radiation perturbs prostaglandin E and F levels, causes an increase in lysosomal enzyme activities, and releases hydrolases from lysosomes in rat tissues. Within 3–6 hours after exposure of rats to a lethal dose of gamma radiation, both the spleen and liver displayed increases in prostaglandins E and F concentrations (figures 5 and 6) and release of hydrolases from their lysosomes (figure 4). Pryanishnikova *et al.* (1978) have observed similar increases at 3 hours postirradiation in the levels of a prostaglandin-like function isolated from mouse spleen and liver tissue. They further found a 40 per cent decrease in concentration of this prostaglandin-like fraction 1 hour after ^{60}Co exposure.

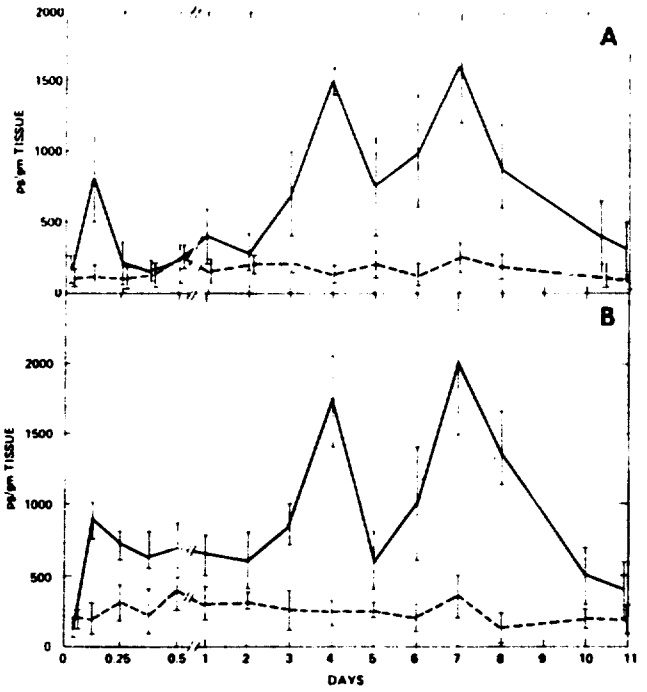

Figure 6. Effect of ^{60}Co gamma radiation on the prostaglandins in spleen. PGE values illustrated in (A); PGF concentrations shown in (B). ——=experimental; - - - - -=control. Means ± S.E. $p \leq 0{\cdot}05$ (toward corresponding spleen control in (A) for 0·125-day and 3- to 8-day PGE samples. $p \leq 0{\cdot}05$ (toward corresponding spleen control in (B)) for 0·125-day to 8-day PGF samples. $n = 12$.

Investigations by others concerning the early effects of radiation on lysosomes agree with our results showing leakage of enzymes from lysosomes 2–6 hours after exposure (figure 4). Hartiala, Nantö, Rinne, and Savola (1960) have reported that a 30 per cent increase in acid phosphatase activity in rat liver lysosomes occurs 6–12 hours following radiation treatment.

As our experiments show, radiation injury of the cell also produces an increase in prostaglandin E and F concentrations and a release of lysosomal enzymes several days after exposure, indicating that these components are interrelated. Previous studies by Eisen and Walker (1976) and Păusescu *et al.* (1976) have also found slight to moderate increases in prostaglandin-like materials in rat and mouse tissues at 3–4 or 7 days after irradiation. The finding by Snyder (1977) and René, Darden, and Parker (1971) showing that lysosomal enzyme release is maximal on days 3–4 and 7–8 also agrees with the results shown in our experiments.

Prostaglandin levels and release of hydrolases from lysosomes might additonally be related to the radiosensitivity of certain organs. For example, the increase in prostaglandin E and F levels and release of hydrolases from the lysosomes were quite large in the spleen, but slight or no elevations for the prostaglandins and leakage of lysosomal enzymes were observed in the radioresistant liver 3 and 8 days postirradiation. However, during early time periods following radiation exposure (3–6 hours), the release of lysosomal enzymes was observed mostly in spleen tissue, whereas, the prostaglandin levels increased by nearly the same amounts in both spleen and liver. Thus it is concluded that during the early time periods there was a correlation of radiosensitivity between only the tissues and the leakage of hydrolases from the lysosomes.

4.2. *Factors responsible for release of lysosomal enzymes and increased prostaglandin levels*

The decrease in size and weight of the spleen that occurs within hours after exposure to ^{60}Co gamma radiation is mainly due to pyknotic changes and death of the highly radiosensitive lymphoid cells. Phagocytosis of these injured cells by macrophages and reticulum cells reaches maximal activity 3 hours after exposure in the spleen (Jordan 1967). The phagocytic process is complete 1 day after exposure. At this time, the spleen has lost 70–80 per cent of its weight. This process, as well as release of enzymes from the disrupted lysosomes, could be responsible for the abrupt rise of prostaglandins 3 hours after irradiation, since Higgs, McCall, and Youlten (1975) have found that white blood cells engaged in phagocytosis release prostaglandins, which then attract more phagocytes. However, the highest prostaglandin levels and greatest release of lysosomal enzymes occurred several days after radiation exposure. By this time, the phagocytic activities have ceased and the spleen has started to regenerate (Jordan 1967). Consequently, there must be other agents that are responsible for the secondary increases in prostaglandin levels and release enzymes from the lysosomes. Eisen and Walker (1976) have observed that the elevation in prostaglandin levels which occurs several days after irradiation might be caused by a reduction in the rate of prostaglandin degradation. It is indeed possible that several enzymes, such as 15 dehydrogenase or 13,14 reductase which are associated with inactivation of prostaglandins might be altered after irradiation.

Radiation damage could also affect other factors related to prostaglandin synthesis. Recent investigations have found that 2–4 and 7–10 days after irradiation there are fluctuations in cyclic nucleotide levels (Trocha and Catravas 1980), disruption of tight junctions in the ilea of the intestine (Porvaznik 1979), inflammation of numerous interstitial tissues (Walker, personal communication), high levels of endotoxins and/or bacteria in spleen and liver (Walker, Ledney and Galley 1975). The above reports indicate that these cell components may be involved in the observed secondary increase in prostaglandin concentrations and release of lysosomal enzymes after exposure of the animal to radiation. In fact, the bacteria and endotoxins could be the main factors which perturb the cell, causing leakage of lysosomal enzymes and synthesis of prostaglandins by disrupting the cellular membrane.

Therefore, the initial and secondary increases in prostaglandin levels and release of lysosomal enzymes could be attributed to membrane disruption in the following ways: (*a*) breakage of mammalian cells by direct interaction of cellular membranes with gamma rays, or from the free water radicals produced by ^{60}Co exposure; (*b*) secondary rupture of cellular membranes due to their exposure to invading endotoxins and bacteria. Disruption of the membranes by these factors could affect enzymes and lipids which are associated with the synthesis and increase in the concentration of prostaglandins following radiation exposure. Formation of these prostaglandins would then destabilize lysosomal membranes, resulting in the dispersal of lysosomal enzymes throughout the cell.

L'irradiation totale de rats conduit à une perte des hydrolases des lysosomes, une augmentation de l'activité des enzymes des lysosomes, et des changements des niveaux des prostaglandines dans la rate et le foie. Une élévation temporaire de la concentration des prostaglandines E et F et une fuite des hydrolases des lysosomes ont été produites dans la rate aussi bien que dans le foie entre 3 et 6 heures après l'irradiation des animaux. Les valeurs maximum de l'activité des hydrolases, de la teneur en prostaglandines E et F et de la perte des

enzymes des lysosomes ont été aussi trouvées dans la rate 4 jours après irradiation. Par contre, dans le foie, seulement de petites élévations du niveau de la prostaglandine F ont été trouvées à ce moment là. Il y eut une augmentation finale des concentrations en prostaglandines E et F et de la perte des hydrolases des lysosomes dans la rate au cours du 7ème jour après irradiation, suivies d'un retour à des valeurs à peu près normales de 11ème jour. La concentration en prostaglandine F dans le foie était aussi légèrement élevée au 7ème jour après quoi elle a diminué au niveau des témoins.

Ganzköperbestrahlung von Ratten befreit die Hydrolasen der Lysosome, erhöht die Aktivität von Enzymen der Lysosome und verändert das Prostaglandin Gleichgewicht in der Milz und der Leber. Man bemerkt eine vorübergehende Zunahme in der Konzentration der Prostaglandin E und F und eine Lockerung der Hydrolasen der Lysosomen in der Milz und der Leber, 3–6 Stunden nach Brestrahlung der Tiere. Der maximale Wert für die Aktivität der Hydrolasen, der Prostaglandine E und F, und der Enzyme der Lysosome wurde 4 Tage nach Bestrahlung in der Milz der Ratten gesehen; während nur eine kleine Steigerung des Prostaglandin F zur selben Zeit in der Leber zu sehen war. Die letzte Steigerung der Konzentration von Prostaglandin E und F und der Hydrolasen der Lysosome der Milz war am 7.Tage. Normale Werte erscheinen am 11.Tage nach der Bestrahlung. Die Konzentration des Prostaglandin F in der Leben war etwas erhöht am 7.Tage nach der Bestrahlung.

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